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SEGREGATION IN RUSSETED SPORTS OF THE GRIMES APPLE [1]

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INTRODUCTION

Clonal varieties of plants, being vegetatively propagated, are generally considered to be characterized by a high degree of uniformity. Indeed, it is sometimes said that the different individuals comprising them are practically identical with each other and with their parents in form and behavior. Uniformity, however, is a relative term. Some clonal varieties, e. g., the Concord grape, are comparatively uniform; others, e. g., the Boston fern, are much more variable. How uniform, or how variable, are the individuals of a clonal variety is often a matter of considerable importance to the nurseryman and likewise to the producer of fruits and ornamentals, for they are obliged to meet certain standards of uniformity or have their products graded down and suffer consequent losses.

It has been known for centuries that rather striking variations now and then appear in vegetatively propagated varieties. These have been called bud sports or mutations and some of them in turn have been propagated as new varieties. With these bud sports, as with their parents, it has been generally assumed that they are stable and uniform in character. Some studies during recent years, both of well known clonal varieties [2] [3] and of certain bud sports [4] [5] [6] [7], have shown that their variability is greater than has been generally thought. This is especially true of those varieties that are commonly classified

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[2] GARDNER, V. R. STUDIES IN THE NATURE OF THE CLONAL VARIETY. III. PERMANENCE OF STRAIN AND OTHER DIFFERENCES IN TREE SIZE IN THE MONTMORENCY CHERRY. Mich. Agr. Expt. Sta. Tech. Bul. 186, 20 pp., illus, 1943.

[3] GARDNER, V. R., and BATEN, W. D. STUDIES IN THE NATURE OF THE CLONAL VARIETY. II. SELECTION WITHIN A PERICLINAL CHIMERA. Mich. Agr. Expt. Sta. Tech. Bul. 179, 48 pp., illus. 1942.

[4] GARDNER, V. R. STUDIES IN THE NATURE OF THE POMOLOGICAL VARIETY. I. A HETERO-CHIMERIC APPLE SPORT AND ITS VEGETATIVE PROGENY. Mich. Agr. Expt. Sta. Tech. Bul. 161., 14 pp., illus. 1938.

[5] GARDNER, V. R. A STUDY OF THE SWEET-AND-SOUR APPLE CHIMERA AND ITS CLONAL SIGNIFICANCE. Jour. Agr. Res. 68: 383–394, illus. 1944.

[6] GARDNER, V. R., CRIST, J. W., and GIBSON, R. E. SOMATIC SEGREGATION IN A SECTORIAL CHIMERA OF THE BARTLETT PEAR. Jour. Agr. Res. 46:1047–1057. 1933.

[7] NEWCOMER, E. H. STUDIES IN THE NATURE OF THE CLONAL VARIETY. IV. CYTOLOGICAL STUDIES OF BUD SPORTS OF MCINTOSH, STARK AND BALDWIN APPLES. Mich. Agr. Expt. Sta. Tech. Bul. 187. 23 pp., illus. 1943.

as chimeras. A study of a large number of bud sports of clonal varieties of apple, pear, cherry, and pelargonium at the Michigan station during the last 25 years has served to emphasize the variable, rather than the uniform, characteristics of many of them. The behavior of certain bud sports propagated for the first time from the parent limb has been especially useful in throwing light on this question.

DESCRIPTION OF MATERIALS

Among a considerable number of russeted apple sports of normally smooth-skinned varieties in the collection of the Michigan Experiment Station are two of the Grimes. One of these, designated as No. 483, was found as a limb variation in a tree in Oceana County, Mich. This limb was brought to the attention of one of the writers in the twenties. It was observed for several successive years when its fruit was approaching the harvesting stage. Its fruits were thinly covered with russet, though not as distinctly russeted as most so-called russet varieties (e. g., Golden Russet, Roxbury, Pumpkin Russet, etc.). The other, designated as No. 581, was a limb sport with similar characteristics on a tree near Montrose, Mich.

Scions were taken from both of these limb sports and a number of nursery trees were grown from them. Each nursery tree grew from a single scion bud. Five trees of No. 483 and three of No. 581 were set in the orchard in 1932 and 1934 respectively. Some of these trees bore a few fruits in 1936 and 1937, but it was not until 1938 that the crops borne by any of them were large enough to warrant making careful records of the amount and distribution of their russeting.

PRESENTATION OF DATA

When the trees of these russeted Grimes sports bore their first fruits it was noted that there were rather marked differences from tree to tree in the amount of russeting. Some trees bore relatively smooth, others relatively russeted fruits. On some of the trees the fruits throughout the tree were comparatively uniform in the amount of russeting; on others there was considerable variation from branch to branch. (See fig 1.) In one instance this difference between branches was so marked that it was decided to harvest the fruits from them and record the data separately. This particular tree had six main branches, labeled A, B, C, D, E, and F; one of these (D) produced a strong lateral that was labeled D_1, the fruits of which were substantially different from those of its parent branch.

The crops borne by the different trees of these two russeted Grimes sports varied in amount from year to year. In certain years (1938, 1942, 1943, 1944, 1945, and 1946 in the case of No. 483 and 1940, 1942, 1943, and 1946 in the case of No. 581) either the entire crop or random samples of 1 or more bushels were harvested from each tree, and each fruit was examined and its percentage of russeted surface estimated and recorded. In other years, because of the small size of the crops or the limitations of time, such detailed records were not obtained. However, in those instances observations were made and notes taken which serve to substantiate the more detailed records made during the other seasons.

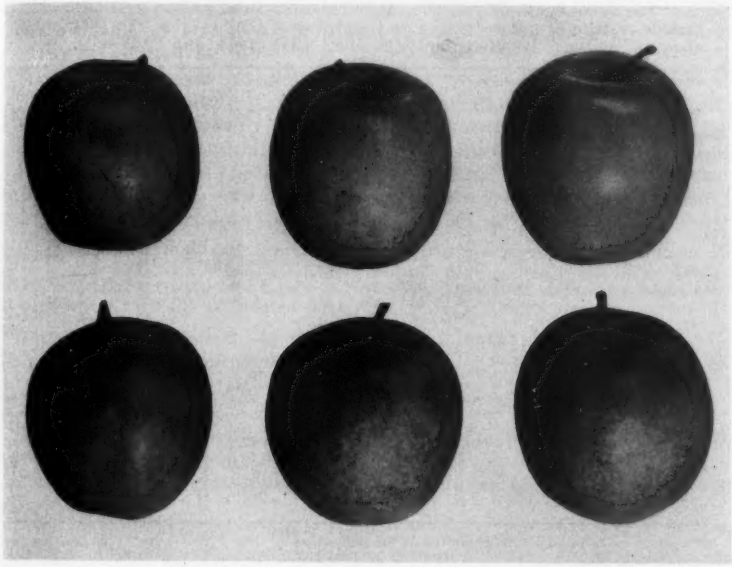

FIGURE 1.—Typical fruits from a single tree of Grimes sport No. 483. These fruits show gradations in russeting ranging from a completely smooth (at the upper right) to a completely russeted (at the lower right) surface.

In tables 1 and 2 detailed data are presented for the 1946 crop season and summarized data for earlier years, showing the percentages of the fruit surfaces covered by russet. These data are presented for each tree of both sports and for each of the several branches of one of the trees of No. 483.

Attention is called first to the marked differences from year to year in the amount of russeting. The seasons of 1942, 1944, and 1945 were characterized by heavy russeting; 1938, 1943, and 1946 were characterized by light russeting; 1940 was intermediate in this respect.

After making due allowance for the influence of season on russeting, it is obvious that different trees of the same selection (i. e., No. 483 or No. 581) not only showed marked differences in the amount of russeting in any one season but these differences continued to manifest themselves year after year. Thus in the case of No. 581, one tree (10S) showed almost complete reversion to the normal smooth skin, a second tree (10R) regularly bore rather heavily russeted fruits, while the third tree (9R) regularly bore rather lightly russeted fruits. The differences between trees 4S, 5S, 3R, and 4R or No. 483 were not so distinct; but those between the different limbs of tree 3S were equally distinct and appeared to be equally fixed. In all of the trees, except 10S (No. 581) and in all of the branches of tree 3S (No. 483) except A, both of which had more or less completely reverted to the parent form, there was rather wide variability in the amount of russeting.

TABLE 1.—Frequency distribution table showing the percentage of russeted surface on fruit of different trees and branches of Grimes sport No. 483 in 1946; mean percentages are given for 1938, 1942, 1943, 1944, and 1945

Percent of surface russeted in 1946	Tree No.										
	4S	5S	[1] 3R	[1] 4R	38, branch						
					A	B	C	D	D_1	E	F
0	27	20	42	129	422	9	4	19	150	6	42
10	25	12	11	16	64	7	26	57	26	9	46
20	91	50	7	23	2	41	56	126	12	64	166
30	170	114	36	75	.....	109	115	145	7	162	236
40	58	45	32	46	.....	60	71	85	7	113	120
50	30	22	59	36	.....	45	45	50	7	67	80
60	24	25	43	22	.....	38	25	26	4	46	43
70	10	3	29	5	.....	17	6	13	.....	17	18
80	8	2	13	2	.....	8	3	9	2	15	10
90	1	4	5	.....	.....	5	4	2	2	4	3
100	1	.....	1	.....	.....	.....	.....	.....	.....	.....	.....
Total	445	297	278	354	488	348	355	532	217	503	764
Mean	31.4	32.6	42.3	20.7	1.4	39.4	35.0	31.2	9.0	39.0	29.7
Means for seasons of:											
1938	36.5	.....	.....	.....	6.4	60.0	55.0	43.6	15.0	37.0	46.7
1942	78.3	88.0	59.7	59.9	10.0	84.5	50.0	59.4	.....	80.4	82.0
1943	31.3	28.8	44.5	44.8	7.3	41.0	21.4	36.0	16.4	46.0	30.9
1944	67.7	.....	.....	.....	18.8	73.4	68.5	60.7	.....	72.5	63.4
1945	73.7	70.2	36.4	65.2	.8	.....	84.5	74.2	.....	81.2	69.6

[1] The apparent bimodal picture presented by the 1946 figures for these 2 trees is due to the fact that both of them, like tree 38, have certain limbs whose fruits show almost complete reversions to the smooth condition of the normal Grimes.

TABLE 2.—Frequency distribution table showing the percentage of russeted surface on fruit of different trees of Grimes sport No. 581 in 1946; mean percentages are given for 1940, 1942, 1943, and 1945

Percent of surface russeted in 1946	Tree No.			Percent of surface russeted in 1946	Tree No.		
	9R	10R	10S		9R	10R	10S
0	.....	.....	198	100	.....	.....	.....
10	35	8	24				
20	91	21	3	Total	310	250	225
30	98	45	.....	Mean	29.8	49.2	1.3
40	39	30	.....				
50	32	42	.....	Means for seasons of:			
60	11	46	.....	1940	58.1	70.4	3.7
70	3	31	.....	1942	94.1	99.7	8.9
80	1	26	.....	1943	41.1	49.6	5.5
90	.....	10	.....	1945	86.5	91.3	.6

DISCUSSION

The data presented suggest that the several trees, or limbs, were perhaps as a whole no more fixed in their character than were the original limb sports from which they were propagated. Obviously the original limb mutations were chimeras (i. e., tissue mixtures); so were some, if not all, of the trees propagated from them. Buds taken from any one of them might yield trees essentially the same as the parent or they might show the diversity that characterizes their own vegetative generation. In their individual tree uniformity and fixity and also in their variability from tree to tree they resemble the sectorial chimera strains of the striped Bartlett pear described by Gardner,

Crist, and Gibson [8] and the heterochimeric Graham apple described by Gardner [9]. Yet on the basis of casual observation these trees would readily be classed simply as a russet variety.

Careful observations and many detailed records that have been made of bud sports of many kinds (color, size, shape, etc., many of which cannot be quantitatively measured or accurately classified as a russeted surface) in the Michigan station's collection suggest strongly that a large proportion of them are of the same basic nature as the two russeted forms here described. This in turn strengthens the practical intrepretation placed on data presented in an earlier report,[10] namely:

> . . . both nurseryman and grower should realize that there are strain differences . . . and nurserymen should propagate and growers should plant those strains whose superior growing and producing qualities have been definitely established as a result of carefully conducted trials.

To this may be added the statement that intravarietal variability is greater than has been generally assumed.

SUMMARY

The characteristics of daughter trees propagated from russeted limb sports of two Grimes apple trees are described.

Some of these daughter trees showed reversions to the normal smooth parental type.

Others showed segregation in respect to amount or percentage of russeting. These segregates exhibited a marked degree of permanence or fixity of type.

Certain trees produced individual limbs showing similar reversion and segregation.

Some practical implications of these characteristics of sporting forms are pointed out.

[8] See footnote 6, p. 225.
[9] See footnote 4, p. 225.
[10] See footnote 2, p. 225.

VARIABILITY AND SEGREGATION IN THE GOLDEN RUSSET APPLE[1]

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INTRODUCTION

Acceptance of the concept that the clonal variety is highly uniform and fixed, at least as compard with the seminally propagated variety, has been general, if not universal. Different individuals of the same clonal variety are often referred to simply as different parts of the same individual and are regarded as practically identical.

Studies of a large number of bud sports of certain deciduous fruits that have been under way at the Michigan Agricultural Experiment Station for a quarter of a century have served to draw attention to their relatively great diversity rather than to their uniformity. Several reports have been made (*2, 4, 6, 7*)[2] describing these intrastrain diversities and pointing out their possible significance. At the same time that the bud sports have been under study, corresponding observations have been made of certain of their parent forms. Most of these parent forms are standard well-known *varieties* (*3, 5, 8*). These varieties are obviously different from their bud sports; otherwise the sports would not be recognized as such. On the other hand, when the parent forms have been compared with their bud sports in such basic characters as uniformity or variabilty, some of them appear to be very much like their progency. Data are presented here on studies made on one of these varieties, the Golden Russet apple.

DESCRIPTION OF MATERIAL AND PROCEDURE

The time and place of origin of the Golden Russet apple are not known. It was brought to this country from England in colonial days, and has been widely propagated by many nurseries and extensively planted, particularly in the Northeastern States.

In describing its fruit, Beach (*1, p. 144*) states:

> *Skin* . . . sometimes only partly covered with patches and flecks of russet but more often almost entirely covered with green or yellowish russet, in highly colored specimens becoming golden russet with bronze cheek. *Dots* grayish or

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[2] Italic numbers in parentheses refer to Literature Cited, p. 240.

russet, rather inconspicuous on the smooth skin but on the russet skin often clear pale gray and conspicuously scattered over the base.

Descriptions of other pomologists agree closely with that of Beach. All classify it as a russet variety, with no suggestion of more variability in the amount or percentage of the fruits' surface being russeted than is true of other varieties of its class. There is, however, in the description of Beach and in those of other writers recognition of some variability in this respect.

The trees of this variety at the experiment station, which furnished the fruits for some of the earlier observations and later the scions for producing another vegetative generation of trees, were 60 to 70 years

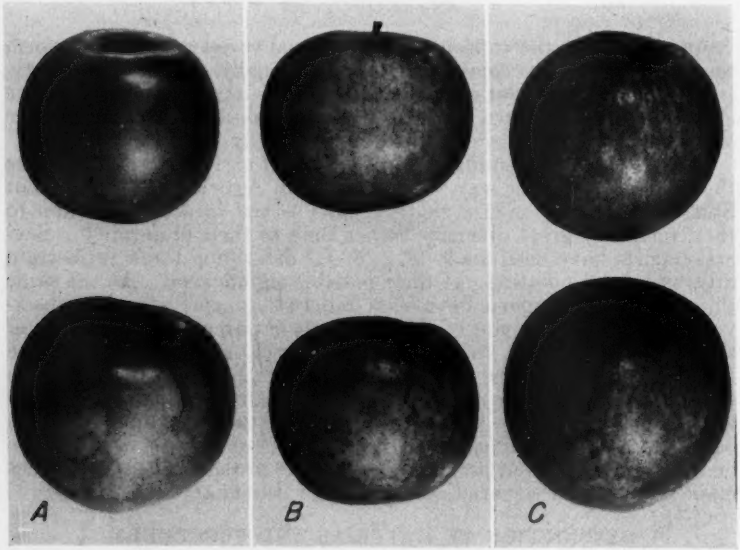

FIGURE 1.—Photographs showing differences in amount of russeting of fruits of the Golden Russet apple. *A*, fruits practically smooth; *B*, about one-fourth russeted; *C*, three-fourths russeted. All fruits are from the same tree

old at the beginning of the study. They appeared to be normal in every respect, similar to thousands of other trees of the same variety in other orchards of the State. Russeting of their fruits could be accurately described by the quotation from Beach in the preceding paragraph. These differences in surface are illustrated in figure 1. It was noted by one of the authors, however, that a large limb of one of these old trees bore fruits that were completely and very heavily russeted, a rather striking bud sport. (See fig. 2.) The occurrence of this limb sport led to close examination of the degree or amount of russeting of fruits borne by other parts of the tree and by other trees of the variety in the same orchard and likewise in a number of other orchards. These observations revealed a wide variation in the amount of russeting on the different fruits, from almost completely covered to almost completely smooth. For the most part these fruits with different amounts of russeting were scattered at random throughout the

trees. Relatively smooth specimens might be borne on spurs next to spurs bearing heavily russeted or semirusseted specimens; or a single spur might bear fruits showing both extremes. Here and there, however, branches of varying size were noted whose fruits seemed to be predominantly rather lightly russeted, or predominantly rather heavily russeted; still others bore fruits showing the entire range in degree of russeting.

It was decided that the observations were interesting enough to warrant a somewhat more extended study. Two large trees in the experiment station orchard at Grand Rapids were selected for it. Five thousand numbered metal tags were fastened to as many individual spurs on these two trees in the fall of 1933 just before harvest.

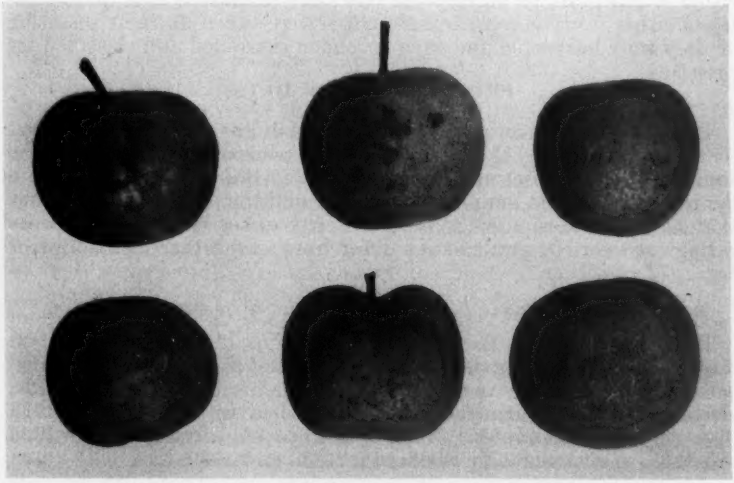

FIGURE 2.—Typical fruits on a limb sport of the Golden Russet apple at the Graham Horticultural Experiment Station, Grand Rapids, Mich. Note the thick, deep character of the russet and the tendency of the skin to crack and scale off

Each apple harvested from a numbered spur was then examined and classified roughly as to amount of russeting by placing it into one of five groups: Completely russeted or practically so, three-quarters russeted, one-half russeted, one-fourth russeted, smooth or almost smooth. Suitable records were made of the character of the fruits borne by each numbered spur. Fruits borne by these same numbered spurs each year for the following 6 years were similarly classified as to amount of russeting and records kept so that one year's performance of a certain spur could be compared with its earlier or later performance and likewise the mass performance of a whole group of spurs on a limb could be compared year after year.

No one spur bore fruits each and every year throughout the 7-year period. Indeed, there were many for which only a single year's performance was available, for there were many breakages, losses of labels, etc. However, there were also many spurs that bore fruit several times during the period in question, enough to furnish some evidence

of the degree of fixity of this characteristic of the fruit in the spur or branch on which it was borne.

Scions were cut from certain limbs selected as producing fruits that fell mainly in one or another of the above classes or that represented certain intermediate conditions. Nursery trees were grown from these scions in the usual manner, each tree developing from a single bud of its scion. Seventy-two trees were set in the orchard in the spring of 1935. The same spring that a part of the scions of these numbered selections were used for bench grafting (1933), others were set as top grafts in already established orchard trees.

Some of these top grafts bore a fairly large number of fruits in 1939 and the orchard trees began producing a year or two later. When either a top graft or an orchard tree bore a considerable number of fruits either fairly comprehensive notes were taken on their russeting or they were harvested and each specimen examined and classified for russeting.

PRESENTATION OF DATA

The data that have been collected furnish at least partial answers to three questions: (1) The influence of seasonal conditions on the amount of fruit russeting, (2) the branch to branch variation within the tree in respect to amount of russeting and the permanence or fixity of those differences, and (3) the possibility of segregating and propagating intravariety strains that differ from each other in amount or degree of russeting.

INFULENCE OF SEASONAL CONDITIONS ON AMOUNT OF FRUIT RUSSETING

Table 1 summarizes the data for the seasons 1933–39, inclusive, on the amount of russeting on the fruits of each of the two parent Golden Russet trees, in the experiment station orchard at Grand Rapids, Mich. In 1933 the fruits russeted rather heavily; in 1934 and 1936 they were almost completely smooth skinned, slightly russeted in 1935 and 1937, and moderately russeted in 1938 and 1939.

TABLE 1.—Mass performance of 2 Golden Russet trees in respect to russeting of fruit for the seasons 1933–39, Grand Rapids, Mich.[1]

Year	Total number of fruits examined for russeting		Mean percent of surface russeted	
	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2
1933	2,060	1,577	43.1	37.8
1934	2,109	1,150	16.0	.5
1935	3,583	4,320	21.5	6.7
1936	1,401	1,721	12.3	.8
1937	2,991	3,750	19.1	6.5
1938	554	1,961	11.6	14.1
1939	[2] 60	16,091	[2] 41.7	18.6

[1] 1936 was a light crop year for both trees. Extremely few records were obtained in 1939 for tree No. 1 because of lack of time.
[2] Sample too small to fairly represent the tree.

TREE, BRANCH, AND SPUR DIFFERENCES IN RUSSETING

There are likewise tree and branch differences that are reasonably consistent from season to season. Thus the furits of tree No. 1 (Table 1) were on the whole more heavily russeted than those of tree

TABLE 2.—Mass performance of selected branches of 2 large Golden Russet trees in respect to russeting of fruit for the seasons 1933-39, Grand Rapids, Mich.

Year	Total number of fruits examined for russeting							Mean percent of surface russeted						
	Tree No. 1			Tree No. 2				Tree No. 1			Tree No. 2			
	Br. 2AA	Br. 5B	Br. 6C	Br. 1B	Br. 2D1	Br. 4A1	Br. 6A	Br. 2AA	Br. 5B	Br. 6C	Br. 1B	Br. 2D1	Br. 4A1	Br. 6A
1933	195	[1] 46	137	[1] 59	96	[1] 20	92	26.5	[1] 99.4	37.0	19.5	11.8	[1] 62.5	47.3
1934	267	139	[1] 27	188	160	[1] 13	126	14.4	98.7	[1] 25.0	0	.2	[1] 0	0
1935	231	115	170	332	183	138	[1] 4	14.1	85.2	12.8	4.1	6.6	11.2	[1] 7.6
1936	90	[1] 6	[1] 2	[1] 44	[1] 4	[1] 4	91	13.3	[1] 83.3	[1] 0	[1] 3.4	[1] 12.5	[1] 6.2	5.5
1937	214	263	124	294	168	126	218	22.9	81.2	6.7	6.3	5.7	6.8	4.2
1938	[1] 38	------	[1] 2	83	90	[1] 49	79	[1] 21.1	------	[1] 12.5	17.8	20.6	[1] 24.5	1.3
1939	[1] 21	------	[1] 2	4,415	694	249	1,372	47.6	------	50.0	6.8	33.0	47.0	11.1

[1] Samples too small to fairly represent the branches.

No. 2. The fruits borne on branches 5B of tree No. 1 and 4A1 of tree No. 2 (table 2) similarly were more heavily russeted than the general tree averages, while branches 6C of tree No. 1 and 1B of tree No. 2 bore fruits less russeted than the general tree averages. In certain seasons these differences were relatively slight. Apparent inconsistencies are probably to be explained by the relatively small numbers of fruits borne by certain limbs in certain seasons—numbers too small to be truly representative of the branch in question.

None of the 5,000 spurs for which records were obtained bore fruits more than 5 times in the 7-year period covered by this study; only a few fruited 4 times; many fruited 3 times. Some of the spurs that bore 3 or 4 times were consistent in producing only smooth or only half-russeted fruits each time; more of them varied from year to year. Thus spur No. 268 produced a smooth-skinned fruit in 1933, 1 that was a quarter russeted in 1935, a smooth one in 1937, 2 smooth ones in 1938, and 1 that was a quarter russeted in 1939. On the whole, individual spur performance in one season gave little indication of what to expect in succeeding seasons. Thus the 346 spurs of tree No. 2 that produced smooth fruits in 1933 produced 69 smooth, 8 quarter-russeted, 2 half-russeted, and 1 fully-russeted fruits in 1935, while the 350 spurs that produced three-fourths russeted fruits in 1933 produced 77 smooth, 20 quarter russeted, 1 three-quarters russeted, and 1 fully russeted in 1935. Their performance in 1936–39 showed similar distributions.

PROPAGATION TRIALS WITH RUSSETING VARIATIONS

When tree branches that appeared to be somehwat different from others in the amount of russeting of their fruits, but whose fruits nevertheless showed considerable diversity in this respect, were put to the propagation test, their daughter trees taken together produced fruits that would classify them simply as Golden Russet. Their fruits were neither more nor less heavily russeted than those of trees of this same variety in other orchards. Closer examination, however, revealed certain rather consistent individual tree and group differences. A large number of detailed records were obtained. Representative data are presented in tables 3 to 5.

TABLE 3.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 485*

Year and tree No.	Number of fruits showing following percent of russeting																				Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
1939																						
Tree S6	6	2	4	2	3	4	2		7	1	2		3	1		7	5	4	8	10	7	78	59.0
Tree S7																						
1940																						
Tree S6																			1	1	20	22	99.3
Tree S7																1		1	2	9	128	141	99.2
1941																						
Tree S6																					6	6	100.0
Tree S7									1		2		2		2		2		6		30	45	91.1
1942																						
Tree S6											1										63	64	99.2
Tree S7															1		5		3	10	404	423	99.9
1944																						
Tree S6													3		6		14		49		175	247	96.1
Tree S7																			36		188	224	98.4
1945																						
Tree A1								1							1		2		5		233	242	99.2
Tree B1																					18	18	100.0
1946																						
Tree A1													1		1		4		50		238	294	97.8
Tree B1															1		20		69		203	293	96.2
Tree C1													2		9		23		46		228	308	95.9

TABLE 4.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 544*

Year and tree No.	Number of fruits showing following percent of russeting																					Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
1941																							
Tree 17A																							
Tree 17B																							
Tree 17C	5	17	21	16	9	7	8	5	5	2	3	1	6	1	3	4	3	1				117	26.3
1942																							
Tree 17A		5			18	6	4		6	6	14	1	1	6		7	1	1	3	6	6	91	49.5
Tree 17B					1	1	1		1	1	1		2			2				2		12	56.8
Tree 17C							4		2			1	1			3	2	2	9	9	13	46	83.4
1944																							
Tree 17A					2		3		3		3		6		5		6		9		14	51	74.5
Tree 17B																							
Tree 17C					1		1		3		11		12		10		9		8		2	57	66.3
1946																							
Tree 17A	13		80		58		39		17		8		13		7		2					237	24.6
Tree 17B						129					34					12					9	184	36.5
Tree 17C	17		63		50		39		21		13		9		8		2					222	25.0

TABLE 5.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 545B*

Year and tree No.	Number of fruits showing following percent of russeting																					Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
1941																							
Tree 2D	1	4										1										6	12.5
Tree 2E	10	14	6	2	2			1	2													37	8.5
Tree 2F																							
1942																							
Tree 2D	3	18	7	11	2	5	5	8	2	5			2		1	4	3	2	1	2		81	26.7
Tree 2E	1	54	2	9	15	3	11	4	10	2			3		1	6				1	1	123	22.7
Tree 2F		13	15	4	4	3		4		2		3	2	2	2	3	1	2	1			63	29.7
1944																							
Tree 2D	1		6		7		8		7		7		7		6		4		4			57	45.4
Tree 2E			6		10		15		19		14		13		8		9		10		9	113	58.5
Tree 2F	1		4		15		12		12		11		14		11		10		6		1	97	49.5
1946																							
Tree 2D	28		71		58		34		25		20		9		10		4		3		2	264	26.2
Tree 2E	85		119		60		12		10		4		1									291	11.8
Tree 2F	17		75		51		27		14		8		3		1		1					197	18.8

The two trees propagated as selection No. 485 from the heavily russeted east limb "sport" rather consistently produced heavily russeted fruits year after year (table 3). Several top grafts of this same selection similarly produced heavily russeted fruits. However, a few fruits borne by these trees and likewise by the top grafts were not entirely covered by russet. Furthermore, hardly any were as thickly russeted as the fruits of the parent limb; their surfaces were completely and evenly covered with a medium thin to thick russet, not the thick, rough covering characterized by checking and scaling pictured in figure 2. No explanation is offered as to why in 1939 tree S–6 of this selection showed such extreme variability in amount of russeting, when it was so much more uniform in this respect in other years.

Each of the daughter trees of selection No. 544 showed wide variation in the amount of russeting of its fruit, but the means for the fruits on each tree were reasonably uniform and were about average for all trees of the Golden Russet variety. Thus they resembled the parent limb from which the scions were taken.

The fruits borne by the three daughter trees of selection No. 545B similarly showed great diversity in amount of russeting, but the percentages of their surfaces covered with russet averaged distinctly lower than those of the daughter trees of selections 485 or 544.

DISCUSSION

The interpretation to be placed upon the data that have been presented on russeting in the Golden Russet apple is that, at least in respect to russeting,[3] this variety behaves like a chimera. This concept would explain not only its great variability but also its tendency to produce segregating limbs and whole trees that exhibit a considerable degree of permanence or fixity in their deviation from the mean or average for the variety. Intravarietal russet strains, comparable to certain chimeral strains of the Graham apple (*2*), Bartlett pear (*6*), and Madame Salleron pelargonium (*5*), originate in the same way. Like them, however, they appear to be rather unstable, ever-sporting strains, whose type can be maintained only by continuous rigid selection. This emphasizes some of the statements made at the beginning of this article to the effect that the Golden Russet apple is characterized more by variability than it is by uniformity.

If the Golden Russet is a chimera, possibly many of the other russeted varieties are likewise chimeras, for observations indicate that they closely resemble it in the matter of russeting. So far as the writers are aware from a review of the literature, russeted apple varieties have not been considered chimeras. They have simply been thought of as ordinary clonal varieties and therefore a priori as characterized by a high degree of uniformity. This raises the question: What percentage of our ordinary clonal varieties are uniform in the sense that they have been so considered and what percentage are in reality chimeras and therefore highly variable?

[3] Observations, in the form of qualitative organoleptic tests, that have been made year after year lead to the belief that it is chimeric in other respects, resembling the Sweet-and-Sour variety described by Gardner (*4*). Smooth-skinned fruits are mild subacid in flavor, somewhat resembling Rhode Island Greening; russeted fruits are sweeter to the taste, spicy, and have a touch of astringency.

SUMMARY AND CONCLUSION

Examination and classification of the fruits borne by 5,000 spurs on 2 Golden Russet apple trees over a 7-year period showed that:

(1) There are large differences in the amount of russeting from year to year, to be attributed to climatic influences.

(2) Individual trees, and likewise individual branches, are characterized by great diversity in any one season in respect to russeting.

(3) In spite of this diversity within branches and within whole trees there are substantial differences between whole trees and between branches of the same tree in respect to russeting of fruit.

Examination and classification of fruits borne over a 7-year period by 72 daughter trees propagated from selected Golden Russet limbs showed:

(1) Varying degrees of diversity within individual trees, comparable to that of the parent trees from which the scion wood was taken.

(2) Individual tree differences and also in some instances differences between groups of trees of the same selection that were more or less constant from year to year.

The data obtained from a study of fruit russeting on both parent and daughter trees lead to the conclusion that the Golden Russet apple variety is a chimera. This suggests that many other ordinary varieties similarly may be chimeras.

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SEGREGATION IN A RADIALLY UNSYMMETRICAL SPORT OF THE CANADA RED APPLE[1]

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INTRODUCTION

Bud sports of fruits that differ from their parents in size are not of common occurrence. They appear now and then, however. In 1936 Shamel and Pomeroy (*8*)[2] stated that they had found references to 30 large-fruited and 2 small-fruited apple sports in their survey of the literature that had appeared up to that time, while they had found 280 references to color variations. Size variations in the grape appear to be relatively more numerous, Shamel and Pomeroy (*8*) listing 56 of this type out of a total of 78 affecting all characters of fruit. A few of the large-fruited forms possess commercial value and have been introduced into cultivation. Among these may be mentioned the King (*3, pp. 324-325*) and Lancaster (*1*) grapes, the Coates (*9, 7*) prune, sports of the Concord, Worden, and Agen varieties, respectively, and the Carapinheira (*4*) pear.

Apparently the fruits of most of these sports are simply larger or smaller, as the case may be, than the normal forms from which they have sprung, resembling them closely in shape, color, flavor, season of maturity, and other characteristics. A few, on the other hand, show a tendency to be irregular or offtype in shape. This irregularity of the few is most likely to take the form of a lack of radial symmetry, evidenced by more or less prominent longitudinal ridges and grooves. Most of the references in the literature to this particular type of variant have been to citrus species, and such terms as "ribbed," "corrugated," and "offtype" have been applied to them. These large-fruited, irregular or offtype sports have been dismissed as curiosities or monstrosities and very few have been propagated.

It has been noted that at least in some instances (e. g., the Karr strain of the Bartlett pear (*10*) limb sports which produce large-sized fruits likewise have leaves larger than those characteristic of the parent variety and sometimes their spurs, shoots, and branches are thicker and more stocky. There is evidence that certain of these giant forms are associated with a tetraploid condition of the chromosomes or other chromosomal abnormalities (*5*).

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[2] Italic numbers in parentheses refer to Literture Cited, p. 255.

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MATERIALS AND OBJECTIVE

Incident to the study of bud sports that has been under way at the Michigan station for many years a considerable number of large-fruited and of small-fruited variants have come under observation. These observations have included such well known varieties as the Bartlett pear, the Wealthy, Duchess, Rhode Island Greening, Grimes, Jonathan, Northern Spy, Baldwin, Delicious, Fameuse, Winesap, and Canada Red apples, and the Hyslop crab apple. Oddly enough, in view of the infrequent references to them in the literature more large-fruited forms have been found that are ribbed or corrugated and that are radially unsymmetrical than of normally shaped radially symmetrical forms. These rogues occur more frequently in certain varieties, e. g., McIntosh and Rhode Island Greening, than in others. Most of these unsymmetrical large- or small-sized sports are so extreme in their irregularity that they are spoken of by the fruit grower as "offtypes" or "rogues." There is reason to believe that they are in reality of more frequent occurrence than casual orchard inspection would indicate, for most fruit growers, upon finding such limb sports or occasionally whole tree sports, promptly remove them or graft them over to something whose fruit is more salable.

One of these aberrant forms, a sport of the Canada Red (Steele Red) apple, was brought to the writers' attention in the early thirties. There were 40 to 50 top-grafted trees of this strain in an orchard near South Haven, Mich., the scions for all of which had been cut from a single tree of that variety some 12 or 15 years earlier. The parent tree had been cut down in the meantime. This entire group of trees bore fruits showing great variation in size and shape. Some fruits were normal in size, some were below normal, and still more above. A few especially of the normal-sized ones, were regular and symmetrical in shape; most of them, especially the undersized and oversized specimens, were irregular and radially unsymmetrical (fig. 1). The trees of the entire group were not examined thoroughly and systematically limb by limb, to determine whether there was a single branch somewhere that bore exclusively undersized or normal or oversized fruits or exclusively regular- or irregular-shaped fruits. However, no such branches were observed. There seemed to be a more or less random distribution of fruits of all sizes and shapes on each and every tree and likewise on each and every top-grafted limb (5 to 10 per tree). Here and there in practically every tree single specimens were noted that were large-sized and radially symmetrical or practically so. It is emphasized, however, that these were isolated and scattered, not segregated in groups.

The lack of radial symmetry of these fruits was extreme, and yet it followed a pattern or series of patterns. Each radially unsymmetrical fruit presented more or less the appearance of a composite of 1 to 4 segments (corresponding to carpels) of large size combined with 4 to 1 segments of normal or of small size; the large radial segments might be adjacent to each other or 2 such segments might have a normal-sized or a small segment between them. Theoretically 39 combinations of such size elements are possible in a 5-carpelled fruit. To the eye some of these combinatins resemble each other rather closely; some are much more extreme than others. Actually how many of them occurred or

FIGURE 1.—End and side views of four typical fruits of Canada Red selection No. 526. All are of giant size for this variety, about 1½ times the size of the normal fruit, and all are irregular in shape and radially unsymmetrical.

the relative frequency of certain of them is not known. Furthermore, normal unequal growth of the 5 segments of a radially symmetrical fruit is great enough to make exact identification of all the classes of irregularity impracticable, if not totally impossible. Suffice it to say that a large percentage of the fruits were radially unsymmetrical and more of them distinctly so than obscurely so.

The fact that some of the fruits borne by these Canada Red trees were less irregular and unsymmetrical to the eye than others, and more especially that a few appeared to be entirely regular and symmetrical,[3] suggested the possibility through scion selection and propagation of segregating out from the parent stock a line or strain uniform for large size—i. e., with all five segments or carpels of the fruit of large size—and consequently radially symmetrical. Of course the same possibility was presented of segregating out a uniform normal-sized, radially symmetrical and a uniform small-sized, radially symmetrical strain.

[3] Assuming random or chance combinations and juxtapositions of large, normal, and small sizes to make up the 5 segments or carpels, if all basic elements are present and have equal opportunities to combine in one way as well as another, 1 fruit out of every 81 could be expected to be regular and symmetrical and 1 out of every 243 could be expected to be large sized, regular, and symmetrical.

Scions (numbered as selections 526 to 532, inclusive) were taken from seven of the Canada Red top grafts and used for production of whole trees and top grafts at the Graham Horticultural Experiment Station. Sixteen top grafts and 21 whole trees of these selections were grown to fruiting age.

Fruits were first produced by some of the top grafts in 1937. Altogether 10 years' fruiting records were obtained at the time of the preparation of this report, though in some seasons the crops were so small because of spring frost damage or other factors that records consisted only of brief notes. However, in those years when any of the top grafts or whole trees produced a considerable number of fruits each fruit was weighed to the closest 10 gm. and was classified as regular and symmetrical in form or as irregular and radially unsymmetrical. In some seasons only certain of the top grafts or certain of the whole trees bore enough fruits to make such detailed records seem worth while. Enough records were obtained, however, or what was regarded as representative tree performance to make certain comparisons.

PRESENTATION OF DATA

The performance of the individual top grafts and the whole trees of the several selections can best be presented in the form of a series of frequency distribution tables showing fruit sizes as determined by weight and the classification of the same fruits as regular or irregular in shape. In the matter of weight exact data were obtained; classification as to regularity in form involved judgement, and different individuals were employed for this work in different seasons. Furthermore, as stated earlier, fruits that obviously should be and were classified as regular were not in fact absolutely regular; the cross sections of few of them would be perfect circles. Certainly out of every hundred normal ungraded fruits of almost any variety, taking them just as they are harvested, there are likely to be a dozen that are more or less irregular and unsymmetrical, perhaps because of insect or fungus injury, abortion of seeds in one or more of the seed cavities, or other factors. In some seasons the percentages of such normally irregular fruits were small; in others they might run as high as 15 to 20. Doubtless, some specimens that should have been classified as irregular were actually classified as regular; probably a still larger number were classified as irregular that should have been classified as regular.

Fruits of selection No. 587 were weighed and classified as to regularity and used as a check on selections 526 to 532. Scions for selection 587 had been obtained from Connecticut and were supposed to be of an especially large-sized strain of the variety. Actually this supposedly large-sized strain proved to be normal for the variety. It will be noted that in 1946 a considerable number of the fruits of this normal stock were classified as irregular in shape (table 1). Those top grafts and trees of selections 526 to 532 that did not produce relatively more irregular-shaped fruits than the trees of No. 587 may be regarded as producing normal-shaped fruits.

TABLE 1.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 587 (check), seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		7-6-D		6-I		7-I		6-J		7-6-B		7-6-D		6-I		7-I		6-J		7-6-B		7-6-D		6-I		7-I		6-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
20																											1			
30											1														1	1			1
40											4														2		3		2	
50							1				4	1													12	1	2		5	
60					2						8	1													10	3	15		2	
70			1		5						28		1				1		1						30	1	37		13	
80	4	1			7		2		2		48		4				2		1						35	2	39		3	
90	7		1	1	9		7		2		71		7				1								40	2	70		5	
100	8	1		2	8		12		6		53	1	14		3		2		1						34	1	85		7	
110	1		2	1	9		6		8		51		14	1	5		10		7						24	2	82		3	
120	9	1	2	1	7		10		7		36		15		10		7		6						9		63		2	
130	1		2		4				2		18		13		4		14		15						5		47		1	
140	1		2		1		3		7		7		5		6		9		14	1					6		30		1	
150	1		2						1		3		4		9		11		14						1		15			
160													1				9		20								9			
170			1								1		2		5		2		11								3			
180											1				2		4		4								4			
190															3		6		6											
200																	3													
210																														
220																	2													
230																	1													
Total	32	3	13	5	50		41		35		334	3	80	1	47		84		100	1					346		210	13	504	45
Mean	105	100	124	104	103		105		116		97	70	116	110	138		144		147	140					99		89	75	116	80
Mean of all (weighted)	108										116										104									

TABLE 2.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 526, seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		7-6-D		1-G		1-H		1-J		7-6-B		7-6-D		1-G		1-H		1-J		7-6-B		7-6-D		1-G		1-H		1-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
20																										4				
30												1														3				
40												3													1	1				
50		3							3			10			1										1	7	1			2
60		6							7			17	1		6										2	6	1			
70		24		4		2	1		11			38	1		5										2	16			1	1
80	2	34		4		4	1		7			33	2		7				1						2	7			1	1
90		34		5		3	2		8			30			16		2								4	13	1		2	1
100		20		2		3	3		3			20	1		9		3		1						5	18	3		1	3
110		10		2		2	4					16			5		2								10	3	3		2	20
120		4		1		1	8					13			4		1		3						12	14	9		1	25
130						1	6					8			1	1	1		2						5	5	10		4	17
140							4								1	1	3		5						11	3	19		4	14
150							4					1			1	3	1		9						5	3	25		2	7
160							1					1				1	2		13						4	3	30		2	8
170																3	2		14						5		31		2	3
180							1									2	2		10								15		2	
190																2	1		10								14			
200																			7								8		2	1
210																			5								7		1	1
220																			5								4			
230																			4								1			
240																			2								2			
250																			4											
260																											1			
270																			1											
280																														
290																			1											
300																											1			
Total	2	135		18		16	35		2	39		191	5	56	10	21	96			7					68	103	186		26	105
Mean	80	86		88		94	123		65	75		88	78	93	165	114	180			114					112	93	165		142	127
Weighted mean	90										116										133									

TABLE 3.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 527, seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		2-G		2-H		2-I		2-J		7-6-B		2-G		2-H		2-I		2-J		7-6-B		2-G		2-H		2-I		2-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
30																									6					
40																							1		15					
50											1														18	3				
60					1	1			3		4												1		25	1		1		
70			2				1		10		3														31		1			
80	2	1	5				2		13		9		1										1		26	2	1			
90	3		11				6		14		13												3	1	11	5	1			
100			21	1	1		5		3		14												6		25	2	7	1		2
110	8		21				2		5		25		1				2						13		26	2	4		1	
120	2		10	1			4				16						2						21	1	11	1	12		2	
130	1		6	1							12		4				3						47	2	25		14	1	7	
140			3	1			2				12		1				5						56	1	16	1	32	7	9	
150											5		1				2						51		6		24	1	4	1
160											6		1				1						32		8		35	2	7	1
170											1		1				3						36		3		32	1	3	
180													1				3						17		1		27	2	2	
190																	2						2	2	1		19			
200													2										6				11		2	
210																	2						7				5			
220													2										3				5			
230													1										1				6			
240																														
250																											2			
260																											1			
Total	16	1	79	4	2	1	22		48		121		16				25						304	7	254	17	239	16	37	4
Mean	105	80	106	123	80	60	102		84		111		156				154						147	141	97	88	163	142	149	127
Weighted mean	99										122												135							

TABLE 4.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 528, seasons of 1941, 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1941; tree—								1943; tree—								1944; tree—								1946; tree—							
	7-6-B		7-6-D		3-G		3-H		7-6-B		7-6-D		3-G		3-H		7-6-B		7-6-D		3-G		3-H		7-6-B		7-6-D		3-G		3-H	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
40																			4											1		
50									1										4										7	2		
60									12										9										13	7		
70									25	1	3						6		18	1									37	4	1	
80	4		2						58	3	2	1	2				8		31	2	2								57	9	2	1
90	1		3						65		2	3	2				15	1	50		1								49	5	6	2
100	5		3						56	1	8		3				21		98	1	5								62	5	13	2
110	6		1						44	1	13	3	3				18		80		1								36	5	27	2
120	8		12	1					18		16						16		61	1	5								14		17	2
130	15	1	7						9	2	11						14		33	1	7								6	1	10	1
140	14	1	15						4		6						11		29	1	6										10	
150	16	1	11						1		6						5		11		8	1							2		7	1
160	8		10								3						5		3		8										3	
170	10		11								1						4		1		4										1	
180	5		13														1				4											
190	10		3								1								1		3											
200	2		5								1										2	1										
210																					1											
220																																
Total	104	3	96	1					293	8	73	7	10				124	1	433	7	57	2							283	39	97	11
Mean	144	140	151	120					90	97	122	97	97				114	90	106	103	147	175							89	82	118	109
Weighted average	147								97								111								97							

TABLE 5.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red Selection No. 529, seasons of 1943, 1944, and 1946*

[R=Regular in shape; I=Irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		7-6-D		4-G		4-I		4-J		7-6-B		7-6-D		4-G		4-I		4-J		7-6-B		7-6-D		4-G		4-I		4-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
40																									2					
50											1		3												6					1
60											2		6												15	1				
70			1				2				8		16	1											23				1	1
80	1						2				13		13	1											39	3	2	1	2	
90							2				24		27						1						38	2	4	2	3	
100	2		6				4				14		16												44		7	3	9	
110	9		4				3				21		30						1						31	2	7	2	14	2
120	1		9				1				15		28												17	1	20	2	36	2
130	3		9								5		15												17		30	2	35	2
140	2		11								3		10	1											3		36	5	48	3
150	3		6								2		8						1						5		38	2	38	1
160			5										5						1						2		34	2	47	1
170			2										3						2						1		10	3	36	1
180			4										1														14	1	26	
190																											7	1	15	
200																											4	1	8	
210																											2		8	
220																											1	1	4	
230																														
240																														
250																														
260																														
270																														
280																													1	
Total	21		57				14				108		181	3					6						243	9	216	28	331	14
Mean	120		134				95				100		107	97					142						95	91	146	139	150	124
Weighted average	125										105										131									

TABLE 6.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 530, seasons of 1941, 1943, and 1944*

[R=regular in shape; I=irregular]

Weight (grams)	1941; tree—				1943; tree—				1944; tree—			
	7-6-B		7-6-D		7-6-B		7-6-D		7-6-B		7-6-D	
	R	I	R	I	R	I	R	I	R	I	R	I
40									1		1	
50					1				3		3	
60					1				10		2	
70					3		3		16		15	
80					6		2		37		23	
90			2		13		15		44		27	2
100	2		4		19	1	14		52		30	
110	3		2	1	17		18	1	50		23	
120	5		5		13	1	14		31		20	
130	1	1	12		6	1	10		18		11	
140	2		8	1			7		9		7	
150	2		10				2		4		3	
160	7		10						3		3	
170	3		6						1			
180	1		1									
190	1		1									
200	3											
210	2		1									
220			2									
Total	32	1	64	2	79	3	85	1	279		168	2
Mean	152	130	137	125	95	117	109	110	101		101	90
Weighted average	142				102				101			

TABLE NO. 7.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red Selection No. 531, seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—												1944; tree—										1946; tree—							
	7-6-B		7-6-D		5-G		5-H		5-I		5-J		7-6-B		7-6-D		5-G		5-H		5-I		5-J		5-G		5-H		5-I		5-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
20																																1
30																																3
40			1					2						2		3										1						8
50		1	1	1			4	4		1						5	1									1	2			1		10
60			1	1		1	6	1				1		3		10										5	4				7	6
70		2	1	9	1	3	6	4						7		8	1						1			14	2			6	6	7
80		4	2	11		4	1	1		2				17		9		3			1	1			2	37	4			16	14	8
90		2		18	1	12	4	1						21		10		7			1		1		2	43	7		1	26	14	4
100		2	3	14		10	3				1			3		10		9				1			1	59	3		2	39	8	1
110		1		11	1	11	3							4		8	1	7								66	7		2	43	6	2
120	1	3		2		5					2			4		3	1	4							5	56			3	40	2	1
130						6								1		7	2	3							3	34	3			32	2	
140														6		1		5			1		2		6	27	1		2	30		
150														1		2	1	4							3	27	2		1	14	1	
160																1	1	1							2	14	3			10	2	
170																		3			1		1			13	5		1	4		
180																										4	1			4	1	
190																	1	1								2	1			5		
200																																
210																														1		
Total	1	15	9	67	3	52	27	13		3	3	1		69		77	9	47				4	2	5	24	403	45		12	271	63	51
Mean	120	91	75	91	90	99	76	61		73	113	60		96		89	99	119				120	90	122	138	115	112		122	119	93	63
Weighted average	93												100												111							

TABLE No. 8.—Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 532, seasons of 1943, 1944, and 1946

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—						1944; tree—						1946; tree—					
	7–6–D		6–G		6–H		7–6–D		6–G		6–H		7–6–D		6–G		6–H	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
30																		1
40															4			1
50															6	2		2
60							1								11	3	1	
70			1			1									19	1	2	2
80	1			1			1								31	3	7	4
90			2				2								52	2	6	5
100	1		1				4		1		1				68	3	35	5
110							2		1		6				52	3	36	10
120					2		1		6		8				37	3	37	7
130							1				7				29	2	18	10
140			1				1		3		3				22	1	27	6
150							2		1		9				8	1	15	1
160							2		2		7				4		13	3
170							1		3		6				1		8	1
180									2		1						4	1
190									4						1		2	
200									2		1						1	
210									1		1						1	
Total	2		5	1	2	1	18		26		50				345	24	213	59
Mean	90		98	80	120	70	118		156		143				103	96	123	113
Weighted average	96						142						110					

Some variation in size of fruit was due to environmental influences. Thus 1946 was a year of drought and in general fruit size was unfavorably affected. Of even greater importance in this study was the influence of relative size of crop borne by certain trees or top grafts, heavy production being responsible for smaller than normal size of individual fruits. No attempt was made to classify the crop of each and every tree and top graft each year as light, medium, or heavy and there is consequently no indication in the tables as to whether any one year's record for a tree is for a heavy or light crop year. Some of the apparent inconsistencies in the tables are due to this factor. Because of the larger crops borne in 1944 and 1946 the data for those years are to be regarded as more representative than those presented for earlier seasons. The records for those 2 years, however, must be interpreted only after giving due consideration to the factor of relative load of fruit. Thus the large sizes of the fruits of selection 532 trees 6–G and 6–H in 1944 (table 8) are obviously associated with light cropping and do not indicate the true fruit size potentialities of the two trees. Mean weights of 116 and 104 gm. (table 1) respectively, for the seasons of 1944 and 1946 may be considered representative for young trees of this variety under a clean culture-cover crop system of soil management.

It will be noted that on the whole the trees and top grafts of all 7 selections (526 to 532) produced fruits that in average size were not greatly different from those of the check trees (No. 587, table 1). Closer examination of the data, however, shows some notable exceptions. Thus tree 5–J and top grafts 7–6–B and 7–6–D of selection 531

(table 7) have consistently produced rather small-sized fruits, while trees 5–G and 5–H of the same selection have consistently produced fruits of about normal size. Tree 4–J of selection 529 (table 5), 2–I of selection 527 (table 3), and 1–H of selection 526 (table 2), have consistently borne large fruits, while trees 4–G, 2–G, and top graft 7–6–B of the same respective selections have consistently borne small fruits. In these particular instances the crops were near enough the same size to make comparisons valid. It would appear that both small-sized and large-sized strains have been segregated out of the original stock, strains that are as uniform for their large size or their small size as the normal strain (No. 587).

In respect to regularity in shape, the top grafts and whole trees on the whole have produced a higher percentage of regular, radially symmetrical fruit than the parent stock from which they were propagated. However, certain top grafts and likewise certain trees (e. g., top grafts 7–6–B, 7–6–D, and trees 1–G and 1–J of selection 526 (table 2)) have borne irregular fruits almost exclusively, while certain other top grafts and trees have borne regular fruits almost exclusively. Regularity in shape may be associated with small size as in tree 5–J of selection 531 (table 7 and fig. 2), with normal size as in tree 6–H of

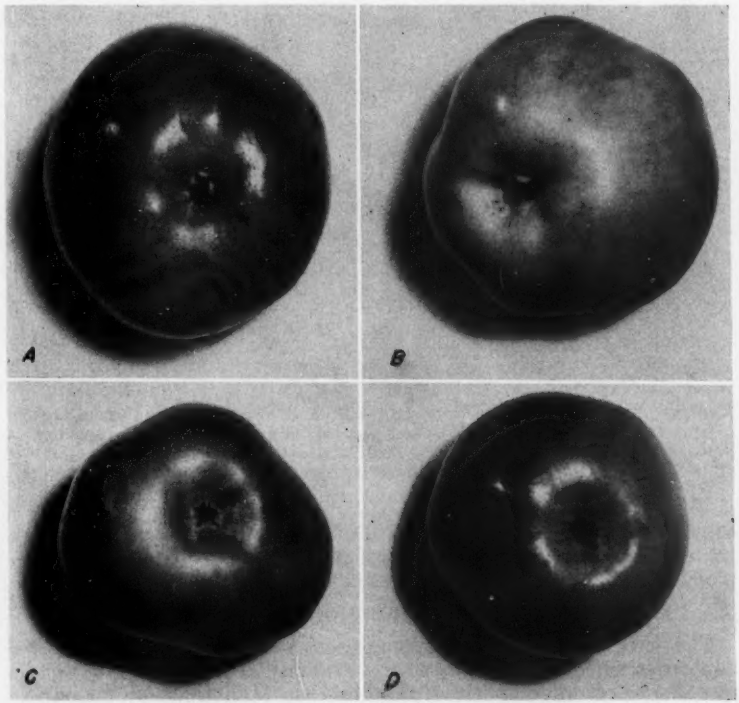

FIGURE 2.—Fruits from tree 5–J of selection 531: *A*, Normal-sized, symmetrical specimen; *B*, normal-sized, radially unsymmetrical specimen; *C* and *D*, small-sized, radially unsymmetrical specimens.

selection 532 (table 8), or with large size as in tree 4–I of selection 529 (table 5). Similarly, regularity in shape may be combined with small size as in tree 4–G of selection 529, with normal size as in top graft 7–6–D of selection 528 (table 4), or with large size as in tree 1–H of selection 526 (fig. 3). From a practical standpoint it is this last

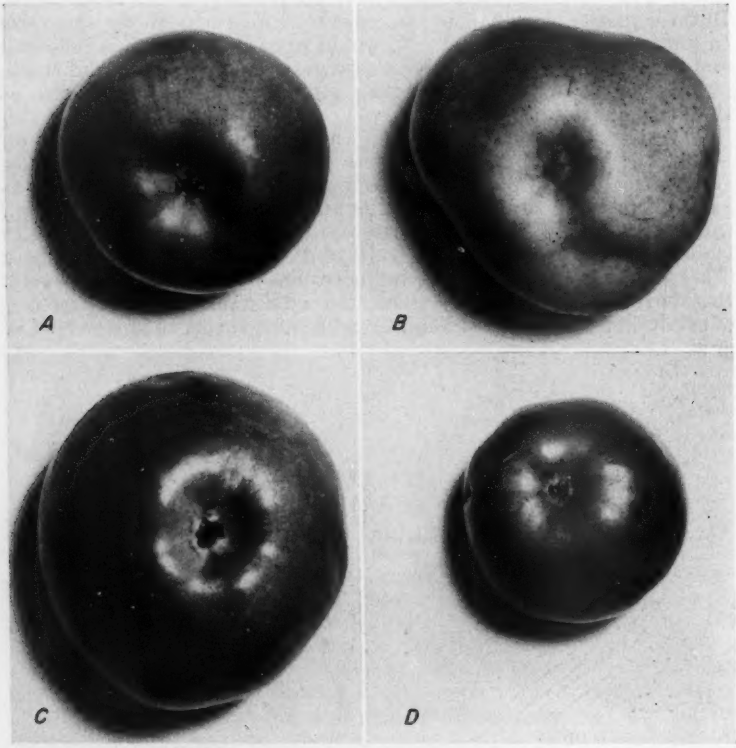

FIGURE 3.—Fruits from selections 587, 529, and 526: *A*, Normal-sized regular fruit from selection 587; *B*, large-sized, radially unsymmetrical specimen of selection 529 from tree 4–I; *C*, a typical large-sized regular fruit of selection 526 from tree 1–H; *D*, small-sized, regular segregate of selection 529 from top graft 7–6–B.

combination for which the apple industry is looking. It appears as though several of the trees and top grafts possess this combination—tree 1–H of selection 526 being perhaps the best representative.

DISCUSSION

The irregular, radially unsymmetrical sports of the Canada Red apple described in this paper are evidently comparable in certain respects to recorded sports of the bizzarria (*11*), Buckeye Navel (*6*), and Thompson Navel (*2*) oranges. These citrus sports are variously classified as sectorial or periclinal chimeras or as due to genic insta-

bility. The descriptions of most of them indicate that they are ever-sporting forms, through at least in the case of the bizzarria (*11*) orange fairly stable segregates have appeared. The Canada Red apple sport would seem to be a sectorial chimera. The two interesting things about it are that: (1) apparently this particular type of sport has not been recorded as occurring in the apple, and (2) out of it has been segregated new comparatively stable strains, at least one of which seems to be promising commercially.

The study suggests that in the case of certain tree fruits and perhaps some of the long-lived, slow-growing ornamentals for which many years are required to obtain a new seed generation, the isolation and propagation of desirable segregates from naturally occurring sports may afford a practicable method of developing new varieties.

SUMMARY

An irregular, radially unsymmetrical bud sport of the Canada Red apple is described, together with its vegetative progeny.

Some of the top grafts and whole trees propagated from this sport closely resemble the parent form. Others show segregation of parental characteristics. Some of these segregates are characterized by a degree of uniformity more or less comparable to that of the parent Canada Red. One of them appears to be worthy of introduction as a new or improved strain of that variety.

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CONTRIBUTION OF INBRED LINES TO THE RESISTANCE OF HYBRID DENT CORN TO LARVAE OF THE EARLY SUMMER GENERATION OF THE EUROPEAN CORN BORER[1]

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INTRODUCTION

In a previous publication[3] certain inbred lines of dent corn (*Zea mays* L.) were shown to be resistant, partially resistant, or susceptible to the survival of larvae of the early summer generation of the European corn borer (*Pyrausta nubilalis* (Hbn.)), and to be able to transmit this resistance or susceptibility to their hybrids. Since there were fewer borers from eggs that hatched on corn in a less advanced stage of growth than from eggs that hatched on corn in a more advanced stage, the population of borers to be expected in each strain was predicted on the basis of the regression of number of borers per plant on silking date.

The inbred lines or corn hybrids that consistently contained fewer than the predicted number of borers were classed as inherently borer-resistant. One experiment involved different combinations of these lines in single-cross hybrids. From borer populations in resistant single crosses averaging 39 percent less than the predicted number of borers, the populations increased to 2 percent more than the predicted number in single crosses made up entirely of partially resistant lines, and to 58 percent more than the predicted number in single crosses involving only susceptible lines. The cumulative effect of multiple factors in inbred lines on borer resistance in hybrids was clearly indicated.

The purpose of the work herein described was to determine whether or not the quantitative effects of the inbred lines in the single crosses were the same as those with double-cross hybrids involving, with two exceptions, the same lines used in the single crosses. Incidental to this study, the possibility of complementary or modifying factors for borer resistance was considered.

STRAINS TESTED

Single-cross hybrids were tested in 1939 and double crosses in 1941, at Toledo, Ohio. The 6 possible single-cross combinations of resistant, partially resistant, and susceptible inbred lines were each

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[2] The authors gratefully acknowledge the assistance of W. A. Baker, under whose general supervision the work was conducted.

[3] PATCH, L. H., HOLBERT, J. R., and EVERLY, R. T. STRAINS OF FIELD CORN RESISTANT TO THE SURVIVAL OF THE EUROPEAN CORN BORER. U. S. Dept. Agr. Tech. Bul. 823, 22 pp. 1942.

represented by 3 to 10 crosses. The crosses involved the resistant lines Ia. L317, Ill. R4, Mich. 77, and Mich. 106, the partially resistant lines Ill. Hy, Ind. TR, Ia. I205, and U. S. 540, and the susceptible lines Ill. A, Ill. 90, Ind. WF9, and U. S. 187–2. Lines L317, R4, Hy, and A in all combinations and crosses on lines TR, I205, 540, 90, WF9, and 187–2 were tested. . In addition, line R4 crosses on 77 and 106, 77 on 106, and 540 on 77, 106, and I205 were tested, making a total of 36 single crosses.

Each of the 15 possible double-cross combinations of resistant, partially resistant, and susceptible inbred lines was represented, with one exception, by 4 double crosses. In order that the combinations made up entirely of resistant or partially resistant lines be represented by more than one double cross, it was necessary to use at least 1 inbred line in addition to the 4 lines involved in the resistant and partially resistant groups of the single crosses. Hence, resistant Wis. CC5 and partially resistant Wis. CC1 were also used in the pedigree of the double crosses. In earlier experiments[4] these lines had about the same effect on borer survival as the other lines in their respective groups, and, as shown in figure 1, a comparison of the single crosses with the double crosses disclosed no significant changes resulting from the use of these lines in the pedigrees of the double crosses. In the combinations made up entirely of susceptible lines, however, another line showing consistent susceptibility to the borer was not available at the time of the experiment. Hence, double cross $(A \times 90) \times (WF9 \times 187\text{–}2)$ was entered 4 times under that combination.

The pedigrees of the 60 double crosses involved the following single crosses: 16 made up entirely of single crosses used in the foregoing test of single crosses; 13 made up with only 1 of the 2 single crosses involving either CC1 crossed on R4, Hy, or A, or CC5 crossed on R4 or A; and 31 made up of 2 single crosses, at least 1 of which was not used in the test of single crosses although they involved the same inbred lines as those crosses. The 31 double crosses involved single crosses of 106 on L317, Hy, and A, of TR on I205, 540, and 90, of WF9 on TR, I205, 540, 90, and 187–2, and of 187–2 on I205 and 540.

The hybrids were designated according to the borer reaction of the inbreds involved. For example, a single cross involving 1 resistant, no partially resistant, and 1 susceptible inbred was designated as a 1–0–1 hybrid combination; and a cross made up of no resistant, 1 partially resistant, and 1 susceptible inbred as a 0–1–1 combination. In double crosses a cross involving 2 resistant, 1 partially resistant, and 1 susceptible inbred was designated as a 2–1–1 hybrid combination.

METHODS AND ANALYSIS OF DATA

The single crosses were grown in 2-hill plots of 3 plants per hill with 7 replications, and the double crosses in 1-hill plots of 3 plants per hill with 8 replications. The hills were 42 inches apart each way. Each plant was infested by hand with 4 egg masses, or about 80 eggs, before the tassels became a factor in borer survival, and each was tagged with the date of silking. All plants were dissected during the last 2 weeks of August to determine the number of borers surviving. The differences in the number of eggs laid naturally on the different hybrids were so small that they were not considered as a factor in the study.

[4] See footnote 3, p. 257.

The first step in the analysis of the data was to eliminate the effect of differences in maturity among the hybrids on the mean number of borers per plant in each hybrid, by use of a regression coefficient determined from previous experiments. The decrease in the mean

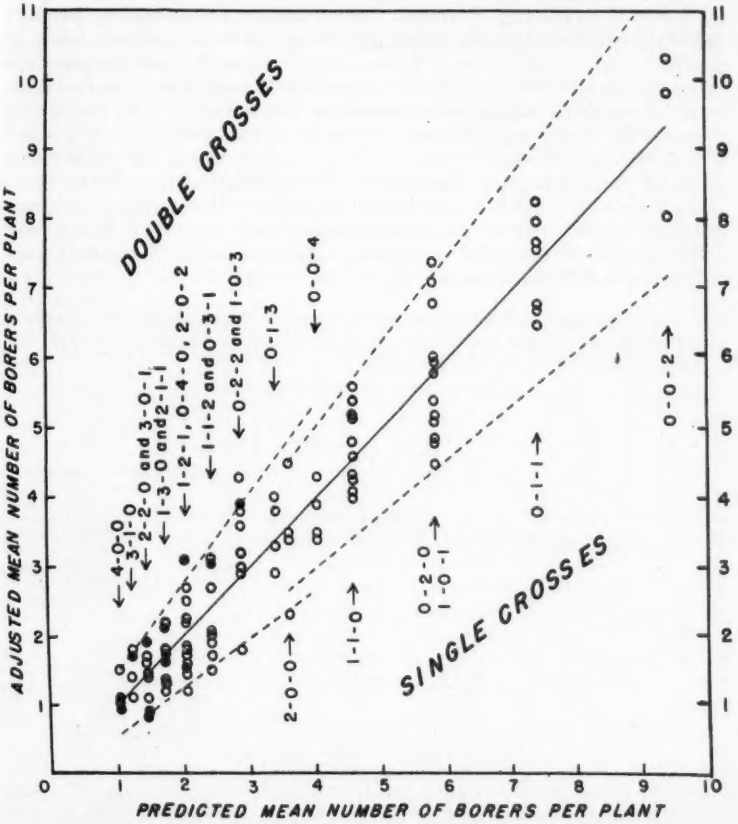

FIGURE 1.—Adjusted mean plotted against predicted mean number of European corn borers per plant in single-cross and double-cross combinations of resistant, partially resistant, and susceptible inbred lines of dent corn. The first, second, and third figures in each set indicate, respectively, the number of resistant, partially resistant, and susceptible inbreds in the different crosses. Open circles indicate different combinations of these lines. Solid circles indicate double crosses in which Wis. CC5 and Wis. CC1 were used. After variability due to replication is removed, 95 percent of the hybrids would be expected to fall within the dotted lines.

number of borers for each day later in silking may be closely estimated by multiplying the mean number of borers per plant in the experiment by 0.043.[5] For each day earlier or later than July 23, the average

[5] PATCH, L. H., and EVERLY, R. T. RESISTANCE OF DENT CORN INBRED LINES TO SURVIVAL OF FIRST-GENERATION EUROPEAN CORN BORER LARVAE. U. S. Dept. Agr. Tech. Bul. 893, 10 pp., illus. 1945.

silking date of the single crosses averaging 6.06 borers per plant, 0.26 was added to or subtracted from the number of borers observed per plant to obtain the adjusted mean. In a similar manner the means of the double crosses that averaged 2.20 borers per plant were adjusted.

The second step was to determine the decrease in borer population in hybrids involving different combinations of resistant, partially resistant, and susceptible lines, as compared with hybrids made up entirely of susceptible lines. In the single crosses the borer populations in combinations 0–0–2, 0–1–1, 0–2–0, 1–1–0, and 2–0–0, were known to decrease from a high to successively lower levels.[6] In the double crosses the borer populations in combinations 0–0–4, 0–1–3, 0–2–2, 0–3–1, 0–4–0, 1–3–0, 2–2–0, 3–1–0, and 4–0–0 would be expected to decrease from a high to successively lower levels if the effects of the inbred lines in double crosses were similar to their effects in single crosses. Certain balanced combinations, such as 1–2–1, 0–4–0, and 2–0–2, would be expected to contain the same level of borers except for the variation due to sampling errors.

TABLE 1.—*Adjusted and predicted mean numbers of European corn borers in groups of single-cross and double-cross combinations of borer-resistant, partially resistant, and susceptible inbred lines of dent corn*

SINGLE-CROSS COMBINATIONS, 1939

1	2	3	4	5	6	7
Prediction group No.	Combination [1]	Number of hybrids in combination	Adjusted mean of borers per plant	Predicted number of borers per plant		
				Arithmetical progression	Geometrical progression	
					Empirical estimates [2]	By method of least squares [3]
1	2-0-0	4	3.43	3.57	3.522	3.611
2	1-1-0	10	4.73	4.67	4.508	4.590
3	1-0-1	8	5.81	5.77	5.77	5.834
	0-2-0	4	5.55	5.77	5.77	5.834
4	0-1-1	7	7.46	6.87	7.386	7.415
5	0-0-2	3	9.38	7.97	9.454	9.424

DOUBLE-CROSS COMBINATIONS, 1941

1	4-0-0	4	1.13	0.97	1.013	1.040
2	3-1-0	4	1.50	1.22	1.106	1.232
3	2-2-0	4	1.58	1.47	1.412	1.460
	3-0-1	4	1.13	1.47	1.412	1.460
4	1-3-0	4	1.55	1.72	1.668	1.730
	2-1-1	4	1.75	1.72	1.668	1.730
	0-4-0	4	2.23	1.97	1.97	2.050
5	1-2-1	4	1.77	1.97	1.97	2.050
	2-0-2	4	1.90	1.97	1.97	2.050
6	0-3-1	4	2.35	2.22	2.327	2.429
	1-1-2	4	2.13	2.22	2.327	2.429
7	0-2-2	4	3.63	2.47	2.748	2.878
	1-0-3	4	3.00	2.47	2.748	2.878
8	0-1-3	4	3.50	2.72	3.245	3.410
9	0-0-4	[4] 4	3.78	2.97	3.832	4.041

[1] Respective numerals indicate the number of resistant, partially resistant, and susceptible inbred lines involved.
[2] The progression ratio is 1.280 and 1.181 for the single and double crosses, respectively.
[3] The progression ratio is 1.271 and 1.185 for the single and double crosses, respectively.
[4] The hybrid (A × 90) × (WF9 × 187-2) was entered 4 times under combination 0-0-4.

[6] See table 11 of reference cited in footnote 3, p. 257.

Column 5 of table 1 shows an arithmetical progression with values that closely approximate the adjusted mean numbers of borers (column 4) of the low-numbered prediction groups, but diverge rather widely from the means of the high-numbered groups. On the other hand, the geometrical progression (column 6) closely approximates the numbers of borers in all the groups. Both the arithmetical and geometrical progressions were determined empirically as giving the best fit to the adjusted data.

For a more accurate determination of the geometrical progression giving the best fit to the data, the method of least squares was employed. The adjusted mean number of borers, Y, in each hybrid was used. With the empirical values of the geometrical progression given in column 6 designated as X, a value of Y' was estimated for each value of X on the basis of the regression of Y on X. The values of Y were paired with the value of X for the group in which they occurred. The values of Y' gave the geometrical progression shown in column 7 of the table. The regression of Y on Y' gave a coefficient of 1.0 borer in each case.

The third step in the analysis of the data was to plot (fig. 1) the adjusted mean number of borers per plant, Y, dissected from the individual hybrids against Y', the number predicted for the group in which they occurred. The regression line was drawn through the plotted points. Then, after the variability due to replication was deducted, the variability from plot to plot within hybrids was determined for 26 levels of borer population by grouping hybrids according to borer level. The data from the double and single crosses fell so closely along the same regression line that both lots were considered as one set. The standard error of the mean of samples of 24 plants containing an average of 1 borer per plant was found to be 0.24, and this value increased by linear regression to 1.46 for samples averaging 9.5 borers per plant. With twice the standard error of the mean of 42 plants for the single crosses and of 24 plants for the double crosses, limits were set off above and below the regression line in figure 1 within which 95 percent of the plotted points would be expected to lie.

EFFECTS OF INBRED LINES IN HYBRID COMBINATION

The combinations of single crosses made up entirely of susceptible, partially resistant, and resistant lines contained estimated averages of 9.42, 5.83, and 3.61 borers per plant, respectively, as compared with 4.04, 2.05, and 1.04 borers in the same combinations of double crosses. There were 61.9 percent as many borers in the partially resistant as in the susceptible combinations of single crosses compared with 50.7 percent in the double crosses. There were also 61.9 and 50.7 percent as many borers in the resistant as in the partially resistant combinations of single and double crosses, and 38.3 and 25.7 percent as many borers in the resistant as in the susceptible combinations of single and double crosses, respectively.

The difference between partially resistant and susceptible, and between resistant and partially resistant, combinations in each case was equal on a percentage basis rather than by an absolute amount.[7] The genes multiply the traits of each other instead of combining additively.

[7] SINNOTT, E. W., and DUNN, L. C. PRINCIPLES OF GENETICS. Ed. 3, 408 pp., illus. New York and London. 1939. See p. 134.

COMPLEMENTARY OR MODIFYING FACTORS

In tests for complementary or modifying factors for borer resistance in an inbred line when used in single crosses, the average reaction to borer survival in single-cross combination with several other lines must be known before it can be determined whether or not that line deviates significantly from the average when in combination with some particular inbred line. For this purpose the data plotted in figure 1 were used. It may be noted that only 2 of the 36 single crosses were outside the limits set by the dotted lines. Five percent, or 2 crosses, would be expected to deviate this much from the prediction line through chance alone. Therefore, whatever complementary or modifying action of factors for borer resistance the inbred lines in combinations may have had was not enough to prove important in 1939 when the variability of the data is considered.

Other tests of the possible effect of complementary or modifying factors for borer resistance were provided by data obtained in 1940 and 1941. In 1940 inbred lines Ia. L317, Ill. M14 and 408, Ind. 38–11, Kan. K226 and K230, Mich. MS1, Minn. A340 and A392, Ohio 02, 07, 51A, and 3113, and Wis. CC5, 3922, and 4308 were tested in single-cross combinations on the common parent lines—susceptible A, partially resistant Hy, and resistant R4. In 1941 the following lines were tested in single-cross combination with Hy and R4: Ia. 159, 289, L304A, L317, BL339, and OS420; Ill. A, Pr, 90, and 5120; Ind. P8, WF9, and 38–11; Kan. K226 and K230; Mich. MS1 and 898; Minn. 49, 50, and 374; Ohio 07, 28, 28A, 33, 40B, 51, 51A, 61–67, and 67A; U. S. 4–8, 153, and 187–2; and Wis. CC2, CC4, CC6, CC7, CC8, and CC11. Single crosses made up of susceptible lines were also included in 1941 as standards for comparison.

In 1940 the number of borers in the 2–0–0, 1–1–0, 1–0–1, 0–1–1, and 0–0–2 combinations averaged 3.886, 4.768, 5.850, 7.178, and 8.807 per plant, respectively, on the basis of a geometrical progression with a ratio of 1.227. In 1941 these combinations averaged 1.664, 2.237, 3.006, 4.040, and 5.430 borers per plant, respectively, on the basis of a geometrical progression with a ratio of 1.344. In 1940 crosses A×51A, A×K226, R4×K230, R4×A340, and R4×CC5 contained significantly fewer borers than the predicted averages of their respective combinations. However, two of these crosses, R4×K230 and R4×CC5, were retested in 1941, and they deviated only slightly, one negatively and the other positively, from the averages predicted. Crosses A×51A, A×K226, and R4×A340 contained 5.5, 5.1, and 2.8 borers per plant, while the average numbers predicted for these respective combinations were 7.18, 7.18, and 3.89 borers. The differences were not more than 0.6 borer greater than those expected on the basis of the within-strain variability. In 1941 single cross Minn. 49×R4 was slightly below the lower limit set up on the basis of the within-strain variability as compared with the expected number. It is concluded that whatever complementary or modifying action of factors for borer resistance the inbred lines might have had in combination with lines A, Hy, or R4, in 1940, or with Hy or R4 in 1941, it was not enough to prove important when the variability of the data is considered.

SUMMARY

The average effect of parent inbred lines of dent corn on the survival of larvae of the early summer generation of the European corn borer in single-cross combinations in 1939 was compared quantitatively with their effect in double-cross combinations in 1941 involving, with two exceptions, the same lines used in the single crosses. The lines used had previously been rated as resistant, partially resistant, or susceptible to larval survival. The tests were conducted by infesting each plant by hand with an average of 80 eggs in addition to the natural infestation, and dissecting the plants later to count the mature borers. From a low population of borers in single crosses or double crosses involving resistant lines, the number of borers per plant increased by geometrical progression in the crosses involving successively more susceptible combinations. On the basis of the progressions there were 38.3 percent as many borers in the resistant as in the susceptible combinations of single crosses, as compared with 25.7 percent as many in the resistant as in the susceptible double crosses. A smaller reduction of borers occurred in the presence of a higher infestation in the single crosses than in the double crosses.

A graphical method for determining the possibility of complementary or modifying action of factors for resistance to borer survival is described. One experiment involving 12 inbred lines indicated no effect of complementary or modifying factors in the 36 single crosses tested. From another experiment in which 16 lines were crossed on susceptible line Ill. A, partially resistant Ill. Hy, and resistant Ill. R4 as the common parents, and from still another experiment in which 39 lines were crossed on Hy and R4, it was concluded that whatever complementary or modifying action of factors for borer resistance the inbred lines may have had was not sufficient to be of importance when the variability of the data is considered.

THE SIGNIFICANCE OF AVAILABLE CALCIUM AS A FACTOR LIMITING GROWTH OF AZOTOBACTER AT pH LEVELS BELOW 6.0 [1]

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INTRODUCTION

The following facts concerning the role played by calcium in the physiologic activity of *Azotobacter* have been established: (1) These organisms are usually absent from soils low in calcium and of low pH value, but are present in soils the pH value of which is appreciably above 6.0, a condition associated with a relatively high calcium content (*4*, *10*) [2]; (2) liming acid soils adequately to maintain a pH appreciably above 6.0 will transform them into a suitable habitat for *Azotobacter* (*11*, *12*); and (3) pure cultures of these organisms will grow abundantly and metabolize atmospheric nitrogen in a modified Ashby medium at pH levels above 6.0, but will not thrive in the same medium buffered to pH values much below 6.0 (*13*, *14*). These facts have been interpreted as indicating a maximum H^+ concentration tolerance of approximately 10^{-6} gram mole per liter by these organisms.

More recently Albrecht and associates (*1*, *2*, *3*, *4*, *8*) have suggested that the lack of available calcium rather than the high H^+ concentration may be responsible for the failure of leguminous plants and their associated nitrogen-fixing rhizobia to thrive in soils of relatively low pH values. It is of interest to know which of these two concepts is correct.

The known low calcium content of acid soils and the depressing influence of phosphates upon the availability of calcium in artificial media under certain conditions suggested that adequate available calcium for *Azotobacter* activity might not be present in a soil, or in a phosphate-buffered medium, such as Ashby's, if the pH is maintained appreciably below 6.0. McCalla (*15*) has called attention to the presence of only 1.29 mg. equivalents of available calcium per liter in one such medium. Fortunately Albrecht and associates (*5*, *6*, *7*) have suggested a method whereby it is possible to increase the supply of calcium available to organisms growing in a medium of low pH value, through the use of calcium adsorbed on collodial clay.

The present investigation was undertaken to find out whether the addition of calcium clay to a modified Ashby medium, adjusted to pH levels at which growth of *Azotobacter* otherwise would not take place would render the medium capable of supporting growth of these organisms. McCalla (*15*) has shown that the addition of collodial clay to

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[2] Italic numbers in parentheses refer to Literature Cited p. 270.

such a medium at favorable pH levels markedly increased the rate of nitrogen fixation by *Azotobacter*, but attributed its value to an over-all increase in the availability of nutrient ions in general, rather than to calcium specifically.

PROCEDURE

Two modifications of Ashby's medium were prepared as follows: Na_2HPO_4 and KH_2PO_4 were mixed in ratios to give solutions buffered at pH values of 5.85, 6.00, 6.20, 6.40, and 6.60. To 2.5 gm. of each of these mixtures was added 0.2 gm. NaCl, 0.2 gm. $MgSO_4.7H_2O$, 0.02 gm. $CaCl_2$, and 0.02 gm. $CaCO_3$.

Medium A was prepared by adding to 1,000 ml. of distilled water, 20.0 gm. of mannitol, 2 drops of 10 percent $FeCl_3$, and 3 mg. of molybdenum as an aqueous solution of MoO_3. To four 125-ml. quantities of this solution (in 250-ml. Erlenmeyer flasks) were added, respectively, 0.368 gm. of the salts mixtured buffered to pH 5.85, 6.00, 6.20, and 6.40. Each of these individual culture solutions contained 1.9 mg. of total calcium.

Medium B was prepared by mixing 150 ml. of a 10-percent suspension of collodial Putnam clay,[3] 250 ml. of saturated $Ca(OH)_2$ (approximately N/25), and water to make 1,000 ml. This was allowed to reach equilibrium, after which 125-ml. quantities for individual cultures were prepared as described for medium A, except that the buffered salts mixtures of 0.2 pH higher values were employed, because it had been observed that the presence of the clay caused a reduction of approximately 0.2 pH in such a medium. Each of these individual culture solutions contained approximately 26.9 mg. of total calcium.

The pH value of each flask of medium was determined with the aid of a Leeds and Northrup glass electrode potentiometer and adjusted if necessary to approximately the desired value, i. e., 5.85, 6.00, 6.20, or 6.40, with NaOH or HCl, after which it was sterilized in an autoclave at 15 pounds pressure. Following sterilization, the pH value was again checked and unless readjustment was necessary these readings were recorded as the initial pH values. These particular pH values were selected because they covered the range within which previous observations had indicated that the maximum tolerance of pure cultures for H^+ concentration might lie, thus providing one or more cultures that would be favorable and one or more unfavorable for growth.

Mannitol-agar slant cultures of *Azotobacter*, grown for 24 hours at 30° C., were washed into a small Erlenmeyer flask containing a few sterile glass beads and thoroughly shaken, in order to secure a homogeneous suspension of cells. Equal quantities of this suspension were pipetted into each flask in a given experiment. A sterile aeration tube passing through a cotton plug was inserted in each flask and connected with the compressed air in a 30° C. incubator. Efforts were made to adjust the flow of air to approximately the same rate in each flask by observing the flow of bubbles. Variations in the flow of air resulting from this procedure appeared to have little effect upon growth as long as bubbling was vigorous.

[3] The dialyzed colloidal clay was prepared in the laboratory of Dr. Wm. A. Albrecht, to whom the writer is indebted for supplying adequate quantities for this investigation.

Thirty minutes after aeration was started samples were taken for the initial count. All counts were made with the aid of a Petroff-Hausser counting chamber and are reported in number of cells per cubic millimeter. The pH values were recorded after growth, but these were of questionable value in many instances, because some strains of *Azotobacter* brought about a rapid and marked reduction in the pH value of the medium while others did not.

The presence of an occasional contamination, resulting from the aeration system, could usually be detected by the microscopic appearance of the cells during the counting. However, purity of the cultures was checked by streaking on both nutrient and mannitol agar. No significance was attached to the growth of *Azotobacter* if accompanied by a contaminant. Although the air was passed through several wash bottles before entering the cultures, prolonged aeration resulted in a decrease in the volume; hence incubation was seldom continued longer than 4 days. There is no reason to think the results would have been different after prolonged incubation.

RESULTS

The complete data from each of 3 experiments conducted with the same strain of *Azotobacter* are presented in tables 1, 2, and 3 to indicate the type of quantitative results secured. Fifteen experiments involving 8 different strains are summarized in table 4. In this table the relative amount of growth, as reflected by cell counts, is indicated by + marks.

TABLE 1.—*Growth of Azotobacter (strain 1d, August 5) at different H^+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 21 hours	Incubation of 26 hours	
	pH of medium	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.80	7,500	6,750	5.80	5,750
Modified Ashby (A)	6.00	7,500	7,750	6.03	6,500
Modified Ashby (A)	6.20	7,500	36,250	6.20	82,500
Modified Ashby (A)	6.38	7,500	237,500	6.40	262,500
Modified Ashby+Ca clay (B)	5.80	7,500	5,750	5.78	6,500
Modified Ashby+Ca clay (B)	6.00	7,500	7,750	6.00	9,000
Modified Ashby+Ca clay (B)	6.20	7,500	38,750	6.20	82,500
Modified Ashby+Ca clay (B)	6.38	7,500	225,000	6.39	187,500

TABLE 2.—*Growth of Azotobacter (strain 1d, September 4) at different H^+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 30 hours	After incubation of 46 hours	After incubation of 70 hours	
	pH of medium	Cell count	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.82	3,750	3,500	2,500	5.83	1,750
Modified Ashby (A)	5.92	3,750	3,250	2,750	6.00	3,000
Modified Ashby (A)	6.25	3,750	3,750	3,500	6.19	34,250
Modified Ashby (A)	6.46	3,750	32,750	94,500	6.39	[1]
Modified Ashby+Ca clay (B)	5.80	3,750	4,000	3,000	5.87	2,500
Modified Ashby+Ca clay (B)	5.96	3,750	3,500	2,750	6.00	2,500
Modified Ashby+Ca clay (B)	6.28	3,750	5,500	50,000	6.18	[1]
Modified Ashby+Ca clay (B)	6.49	3,750	20,000	44,250	6.38	[1]

[1] After heavy growth, counts were unsatisfactory and were not recorded.

TABLE 3.—*Growth of Azotobacter (Strain 1d, September 18) at different H^+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 44 hours	After incubation of 69 hours	After incubation of 93 hours	After incubation of 118 hours	
	pH of medium [1]	Cell count	Cell count	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.85	2,250	2,500	2,000	2,750	5.80	2,250
Modified Ashby (A)	6.00	2,250	2,250	2,250	2,000	6.00	1,750
Modified Ashby (A)	6.20	2,250	3,000	142,500	(2)	5.95	(2)
Modified Ashby (A)	6.40	2,250	245,000	(2)	(2)	6.18	(2)
Modified Ashby+Ca clay (B)	5.85	2,250	2,750	3,000	2,750	5.80	3,250
Modified Ashby+Ca clay (B)	6.00	2,250	2,500	2,500	3,000	6.01	4,750
Modified Ashby+Ca clay (B)	6.20	2,250	3,000	2,750	4,000	6.22	51,250
Modified Ashby+Ca clay (B)	6.40	2,250	3,250	4,250	122,500	6.34	(2)

[1] Approximate pH of medium.
[2] After heavy growth, counts were unsatisfactory and were not recorded.

TABLE 4.—*Relative growth [1] of Azotobacter at different H^+ concentrations in the presence and absence of calcium clay*

Experiment No.	Strain No.	pH range							
		5.70–5.90		5.91–6.10		6.11–6.30		6.31–6.50	
		Medium A	Medium B	Medium A	Medium B	Medium A	Medium B	Medium A	Medium B
1	5	–	–	0	0	–	++	++++	+++
2	A_2	–	–	–	++	?	++++	++++	+++
3	K	–	–	?	?	+++	++	++++	+++
4	d	–	–	0	++	–	0	++++	++++
5	A_2	–	–	–	–	++++	?	++++	++++
6	B_3	–	–	–	–	+	++++	++++	++++
7	A_2	–	–	–	–	–	–	++++	++++
8	K	–	–	–	–	++	++	++++	+++
9	1d	–	–	+++	?	++++	++++	++++	++++
10	M	–	–	?	–	+	?	++++	++++
11	1d	–	–	–	–	+	++++	++++	++++
12	1d	–	–	–	–	++++	++	++++	++++
13	A1	–	–	++	+	+++	+	++++	++++
14	1d	–	–	–	–	++	++	++++	++++
15	1d	–	–	+	++	++++	+++	++++	++++

[1] – No evident growth; ?, questionable growth; +, slight but distinct growth; ++, some growth; +++ good growth; ++++, heavy growth.

It may be recalled that the phosphate salts were mixed to give pH values of 5.85, 6.0, 6.2, and 6.4; however, individual flasks varied somewhat from these values and no special effort was made to adjust them exactly to these points. In table 4 the cultures with various pH values have been grouped under four headings, i. e., 5.70 to 5.90, 5.91 to 6.10, 6.11 to 6.30, and 6.31 to 6.50. In only one instance in this data did the paired cultures of the two media fall in different categories.

DISCUSSION

Rather marked variation was noted in the rate of growth of different strains of *Azotobacter* at comparable pH levels and of the same strain at the same pH value in different experiments, even at the higher and

more favorable pH levels. This variation may have been due, in part at least, to the fact that the time involved in counting cells was so great that only one strain could be grown in a single experiment, and hence identical conditions did not obtain in all experiments. Similar variability in the growth of *Azotobacter* has been observed before.

Differences in the rate of growth might also have resulted in some instances from a retardation of growth coming from a rapid increase in the H^+ concentration produced by some strains and not by others, but this could not account for the differences recorded in tables 1 and 2 since there was practically no change in the H^+ concentrations.

Some differences in the maximum H^+ concentration at which growth took place are evident from a casual study of table 4. In no instance, however, did growth take place below pH 5.9, and with every strain good growth took place at pH values below 6.4. This is the range of pH over which inhibition of growth of *Azotobacter* has been previously reported (*10*, *12*, *13*).

The interesting point in this connection is that the addition of calcium clay to the modified Ashby medium had little or no influence upon the maximum H^+ concentration tolerated by *Azotobacter*. In experiments 1 and 2 growth occured at a somewhat lower pH level in the presence of the calcium clay, while the reverse was true in experiments 5, 9, and 11. Similarly, somewhat more rapid growth took place in the presence of the clay, near the maximum H^+ tolerated, in experiments 6 and 11; the reverse condition existed in experiments 12 and 13. Since the relative growth of *Azotobacter* in the two media in the 15 experiments recorded in table 4 is indicated by the number of + marks, a comparison of a summation of these for the two media in the various pH categories should give a fair over-all measure of the benefit derived from the presence of calcium clay. Such a comparison indicates that the additional calcium carried by the calcium clay did not facilitate the growth of *Azotobacter* at pH levels where it would not grow otherwise.

SUMMARY

Several strains of *Azotobacter chroococcum* were found to grow abundantly in a modified Ashby medium containing 15 p. p. m. of calcium added as $CaCl_2$ and $CaCO_2$ when the pH was appreciably above 6.0. The same organisms would not grow in this medium if it were buffered at pH levels appreciably below 6.0. The addition of approximately 200 p. p. m. of calcium in the form of calcium clay did not transform this modified Ashby solution into a suitable medium for the growth of *Azotobacter* at pH levels below 6.0. These facts are interpreted as indicating that the lack of available calcium is not the factor responsible for the failure of *Azotobacter* to grow at pH levels below 6.0.

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